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Access DB# 87461

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Jon D Eggerton Examiner #: 79431 Date: 2/25/03
Art Unit: 1639 Phone Number 30 8-2423 Serial Number: [redacted]
Mail Box and Bldg/Room Location: CM1-3014 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need. mej

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: See Attached sheets

Inventors (please provide full names):

Earliest Priority Filing Date: 2/12/1999

**For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

See Attached Sheets

POINT OF CONTACT:
PAUL SCHULWITZ
TECHNICAL INFO. SPECIALIST
CM1 6B06 TEL. (703) 305-1954

****

STAFF USE ONLY	Type of Search		Vendors and cost where applicable	
Searcher:	NA Sequence (#)		STN	509.71
Searcher Phone #:	AA Sequence (#)		Dialog	
Searcher Location:	Structure (#)		Questel/Orbit	
Date Searcher Picked Up:	2/26	Bibliographic	Dr.Link	
Date Completed:	2/27	Litigation	Lexis/Nexis	
Searcher Prep & Review Time:	30	Fulltext X	Sequence Systems	
Clerical Prep Time:		Patent Family	WWW/Internet	
Online Time:	79	Other	Other (specify)	

PTO-1590 (8-01)

SEARCH REQUEST FORM

Name:	Jon D. Epperson
Examiner #	79431
Date:	2/25/03
Art Unit	1639
Phone #	308-2423
Location	CMI-3D14
Format	Paper
Serial Number	09/920,435
Title of Invention	Size-exclusion-based extraction of affinity ligands and active compounds from natural samples
Inventor(s)	Yuriy M. Dunayevskiy, Dallas E. Hughes; Andrew S. Weiskopf
Earliest Priority	2/12/1999

Dear Stic,

1) Please do a "key word" search on any relevant terms in claims 1-14 (see attached sheet):

2) Please try to find the earliest "papers", "patents" or "meetings" for the "automated ligand identification system (ALIS)" by "NeoGenesis" (Cambridge, MA, USA) (see attached figure 5) – anything before 2/12/1999 would be very helpful!

Summary of Invention: The invention is drawn to a "method" for screening a library of potential drugs against a target protein wherein size-exclusion chromatography is used "twice", first to separate the "protein-ligand" complex from unbound ligands, and then to separate the ligand from the protein after the protein-ligand complex has been separated by some sort of chemical or physical process e.g., heat, denaturants. The novelty here appears to be that "two separation steps" are being used each employing a "size exclusion" methodology. Finally, the separated ligand is identified by mass spectroscopy and thus a potential drug is found for that particular protein. A picture is provided (see attached figure 5) to give you an idea of what this invention is all about. Please note: it is very important that you search for "two" separation steps using "size exclusion" methods.

Thanks so much for your help!

-Jon Epperson

CLAIMS

What is claimed is:

1. A method of screening a natural sample for an affinity ligand that binds to a protein target, comprising:

(1) mixing a protein target and a natural sample in solution to form a reaction mixture;

(2) incubating the reaction mixture under conditions allowing complex formation by the target and any target-binding ligand present in the sample;

(3) passing the reaction mixture through a first size-exclusion medium that removes from the reaction mixture any small molecular weight compounds each having a molecular weight less than a first preset value;

(4) subjecting the size-excluded reaction mixture from step (3) to conditions promoting dissociation of any ligand/target complex into free ligand and free target; and

(5) passing the reaction mixture resulting from step (4) through a second size exclusion medium that removes from the reaction mixture any molecule larger than a second preset value.

2. The method of claim 1, wherein the first size-exclusion medium removes molecules having a molecular weight of about 2,000 daltons or less.

3. The method of claim 1, wherein the first size-exclusion medium removes molecules having a molecular weight of about 1,500 or less.

09/920,435

4. The method of claim 1, wherein the first size-exclusion medium comprises a gel filtration or size exclusion HPLC column.

5. The method of claim 1, wherein step (4) comprises adding to the size-excluded mixture from step (3), a solution comprising an organic solvent and an organic acid.

6. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium comprises an ultrafiltration membrane.

7. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 10,000 daltons or more.

8. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 3,000 daltons or more.

9. The method according to claims 1, 4, or 5, wherein the second size-exclusion medium removes from the reaction mixture, molecules having a molecular weight of about 2,000 daltons or more.

10. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 10,000 daltons or more.

11. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 3,000 daltons or more.

12. The method of claim 6, wherein the ultrafiltration membrane removes from the reaction mixture, molecules having a molecular weight of about 2,000 daltons or more.

13. The method according to claims 1, 4, or 5, further comprising:

(7) comparing the analytical results of step (6) with a reference standard.

14. The method of claim 13, wherein the reference standard comprises the analytical results of subjecting either a sample of the protein target alone or a mixture of the protein target with a non-target-binding natural sample, to steps (2)-(6).

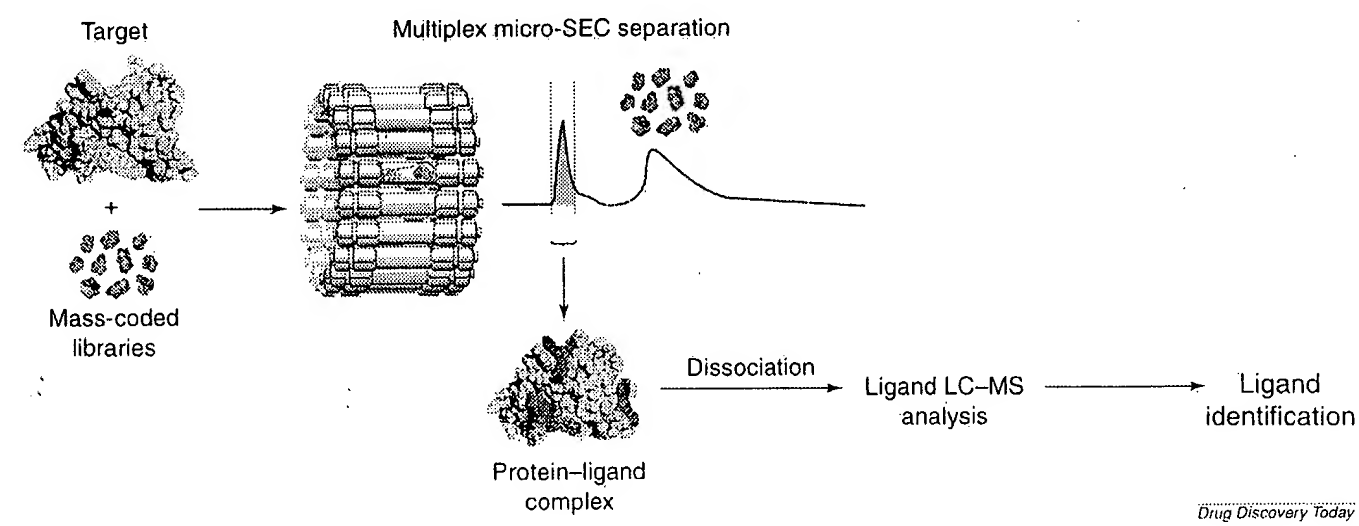
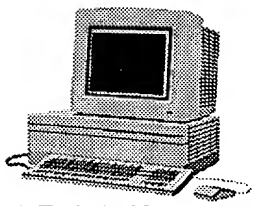


Figure 5. NeoGenesis (Cambridge, MA, USA) integrated ultra-high-throughput small-molecule automated ligand identification system (ALIS). Combinatorial library mixtures are screened by size-exclusion chromatography coupled to reverse-phase HPLC and high-resolution electrospray mass spectrometry. Multi-thousand member library mixtures are designed to be self-encoded by the mass of each compound (mass coded), such that the mass of each ligand directly identifies its composition (building block plus core combination).

BioTech-Chem Library
Search Results
Feedback Form (Optional)



Scientific & Technical Information Center

The search results generated for your recent request are attached. If you have any questions or comments (compliments or complaints) about the scope or the results of the search, please contact ***the BioTech-Chem searcher*** who conducted the search ***or contact***:

Mary Hale, Supervisor, 308-4258
CM-1 Room 1E01

Voluntary Results Feedback Form

- *I am an examiner in Workgroup:* *(Example: 1610)*
- *Relevant prior art **found**, search results used as follows:*
  - ☐ 102 rejection
  - ☐ 103 rejection
  - ☐ Cited as being of interest.
  - ☐ Helped examiner better understand the invention.
  - ☐ Helped examiner better understand the state of the art in their technology.

Types of relevant prior art found:

- ☐ Foreign Patent(s)
- ☐ Non-Patent Literature
  (journal articles, conference proceedings, new product announcements etc.)

- *Relevant prior art **not found**:*
  - ☐ Results verified the lack of relevant prior art (helped determine patentability).
  - ☐ Search results were not useful in determining patentability or understanding the invention.

Other Comments:

Drop off completed forms at the **Circulation Desk CM-1,** or send to Mary Hale**, CM1-1E01 or e-mail mary.hale@uspto.gov.**

```
=> b hcaplus
FILE 'HCAPLUS' ENTERED AT 13:59:08 ON 27 FEB 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)
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FILE COVERS 1907 - 27 Feb 2003  VOL 138 ISS 9
FILE LAST UPDATED: 26 Feb 2003  (20030226/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.


=> d que l58
L10       22776 SEA FILE=HCAPLUS ABB=ON  PLU=ON  DRUG SCREENING+OLD/CT
L11       28281 SEA FILE=HCAPLUS ABB=ON  PLU=ON  LIGANDS+NT/CT
L12      611621 SEA FILE=HCAPLUS ABB=ON  PLU=ON  PROTEINS/CT
L15         892 SEA FILE=HCAPLUS ABB=ON  PLU=ON  SIZE-EXCLUSION CHROMATOGRAPHY/
                CT
L18       84965 SEA FILE=HCAPLUS ABB=ON  PLU=ON  LIQUID CHROMATOGRAPHY+OLD,NT/C
                T
L19       44853 SEA FILE=HCAPLUS ABB=ON  PLU=ON  HPLC+OLD,NT/CT
L20        2671 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ULTRAFILTERS+OLD,NT/CT
L21        5820 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ULTRAFILTRATION+OLD,NT/CT
L23           3 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L10 AND L11 AND L12 AND (L20
                OR L21)
L24          20 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L10 AND L11 AND L12 AND (L15
                OR L18 OR L19)
L25           4 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L24 AND SIZE EXCLUS?
L58           5 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L23 OR L25


=> b medline
FILE 'MEDLINE' ENTERED AT 13:59:19 ON 27 FEB 2003

 FILE LAST UPDATED: 26 FEB 2003 (20030226/UP).  FILE COVERS 1958 TO DATE.

 On June 9, 2002, MEDLINE was reloaded.  See HELP RLOAD for details.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2003 vocabulary.  See http://www.nlm.nih.gov/mesh/summ2003.html
 for a description on changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.
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```
=> d que l37
L30       28179 SEA FILE=MEDLINE ABB=ON  PLU=ON  LIGANDS/CT
L31      115960 SEA FILE=MEDLINE ABB=ON  PLU=ON  PROTEINS/CT
L32       54732 SEA FILE=MEDLINE ABB=ON  PLU=ON  "CHROMATOGRAPHY, GEL"+NT/CT
L33      294236 SEA FILE=MEDLINE ABB=ON  PLU=ON  "CHROMATOGRAPHY, LIQUID"+NT/CT

L35       72167 SEA FILE=MEDLINE ABB=ON  PLU=ON  "DRUG EVALUATION, PRECLINICAL"
                +NT/CT
L37           2 SEA FILE=MEDLINE ABB=ON  PLU=ON  L30 AND L31 AND L35 AND (L32
                OR L33)


=> b embase
FILE 'EMBASE' ENTERED AT 13:59:25 ON 27 FEB 2003
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 FILE COVERS 1974 TO 20 Feb 2003 (20030220/ED)

 EMBASE has been reloaded. Enter HELP RLOAD for details.

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 substance identification.

=> d que l52
L43       14489 SEA FILE=EMBASE ABB=ON  PLU=ON  LIGAND/CT
L44       68430 SEA FILE=EMBASE ABB=ON  PLU=ON  PROTEIN/CT
L45      192037 SEA FILE=EMBASE ABB=ON  PLU=ON  CHROMATOGRAPHY+NT/CT
L46       79759 SEA FILE=EMBASE ABB=ON  PLU=ON  HIGH PERFORMANCE LIQUID
                CHROMATOGRAPHY/CT
L47        3379 SEA FILE=EMBASE ABB=ON  PLU=ON  GEL PERMEATION CHROMATOGRAPHY/C
                T
L48        5272 SEA FILE=EMBASE ABB=ON  PLU=ON  ULTRAFILTRATION/CT
L51      126239 SEA FILE=EMBASE ABB=ON  PLU=ON  SCREENING+NT/CT
L52           1 SEA FILE=EMBASE ABB=ON  PLU=ON  L43 AND L51 AND L44 AND (L45
                OR L46 OR L47 OR L48)


=> b drugu wpix
FILE 'DRUGU' ENTERED AT 13:59:34 ON 27 FEB 2003
COPYRIGHT (C) 2003 THOMSON DERWENT

FILE 'WPIX' ENTERED AT 13:59:34 ON 27 FEB 2003
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=> d que l55
L55           3 SEA PROTEIN? AND (CHROMATOGRAPHY OR SIZE EXCLUS? OR HPLC OR
                GEL PERMEAT?) AND (ULTRA FILT? OR ULTRAFILT?) AND LIGAND AND
                SCREEN?


=> dup rem l37 l58 l52 l55
FILE 'MEDLINE' ENTERED AT 13:59:58 ON 27 FEB 2003

FILE 'HCAPLUS' ENTERED AT 13:59:58 ON 27 FEB 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
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FILE 'EMBASE' ENTERED AT 13:59:58 ON 27 FEB 2003

FILE 'WPIX' ENTERED AT 13:59:58 ON 27 FEB 2003

PROCESSING COMPLETED FOR L37
PROCESSING COMPLETED FOR L58
PROCESSING COMPLETED FOR L52
PROCESSING COMPLETED FOR L55
L59 10 DUP REM L37 L58 L52 L55 (1 DUPLICATE REMOVED)

=> d ibib ab hitind l59 1-10

L59 ANSWER 1 OF 10 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:77049 HCAPLUS
TITLE: Human tissue-specific drug screening procedure and tissue cartridge
INVENTOR(S): Bukusoglu, Cuneyt
PATENT ASSIGNEE(S): Signet Laboratories, Inc., USA
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003008968	A2	20030130	WO 2002-US23138	20020718

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-307062P P 20010719
AB The invention discloses a method of using tissue cartridges contg. one or more tissue samples in a configuration allowing screening of drug candidates against normal or known disease states. The method generates binding information for multiple drug-human tissue sections. This binding information helps identify drug candidates having specific binding characteristics, allowing for selection of potential drug candidates having specific binding characteristics, allowing for selection of potential drug candidates that have the desired binding qualities. The ability to understand binding characteristics allows drug discovery methods that reduce potential side effects.
IC ICM G01N033-50
CC 1-1 (Pharmacology)
Section cross-reference(s): 9
IT AIDS (disease)
Affinity chromatography

Alzheimer's disease
Anti-AIDS agents
Anti-Alzheimer's agents
Anti-infective agents
Anti-inflammatory agents
Antidiabetic agents
Antitumor agents
Antiviral agents
Buffers
Capillary electrophoresis
Chelating agents
Detergents
Diabetes mellitus
Down's syndrome
Drug screening
Electrophoresis
Gaucher disease
Gel permeation chromatography
Human
Human immunodeficiency virus
Human papillomavirus
Human poliovirus
Hydrophobic interaction chromatography
Infection
Ion exchange HPLC
Ion exchange chromatography
Kidney
Kidney, neoplasm
Leukemia
Liver
Liver, neoplasm
Lung
Lung, neoplasm
Lymph node
Mammary gland
Melanoma
Muscle
Myasthenia gravis
Myoma
Neoplasm
PCR (polymerase chain reaction)
Pancreas
Pancreas, neoplasm
Physiological saline solutions
Prion diseases
Reducing agents
Reversed phase HPLC
Reversed phase liquid chromatography
Stomach
Stomach, neoplasm
Thermocouples
Tuberculosis
Tuberculostatics
(human tissue-specific drug screening procedure and tissue cartridge)

IT Antibodies
Carbohydrates
Inorganic compounds

Lipids
Nucleic acids
Organic compounds
Peptides
Proteins
RL: BSU (Biological study, unclassified); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(human tissue-specific drug screening procedure and tissue cartridge)

IT High-performance gel-permeation chromatography
(**size-exclusion**; human tissue-specific drug screening procedure and tissue cartridge)

L59 ANSWER 2 OF 10 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:332620 HCAPLUS
DOCUMENT NUMBER: 136:337372
TITLE: **Size-exclusion**-based extraction of affinity ligands and active compounds from natural samples
INVENTOR(S): Dunayevskiy, Yuriy M.; Hughes, Dallas E.; Weiskopf, Andrew S.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 14 pp., Cont.-in-part of Appl. No. PCT/US00/03562.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002052006	A1	20020502	US 2001-920435	20010801
WO 2000047999	A1	20000817	WO 2000-US3562	20000211

W: CA, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRIORITY APPLN. INFO.: US 1999-119966P P 19990212
WO 2000-US3562 A2 20000211

AB The invention encompasses an improved, rapid, **size-exclusive** method for screening for small mol. wt. ligands that bind specifically to a protein target, using **size-exclusion** sepn., ultrafiltration, and mass spectrometry.
IC ICM G01N033-53
NCL 435007100
CC 9-16 (Biochemical Methods)
Section cross-reference(s): 63
ST **size exclusion** extn affinity ligand active compd natural
IT **Ligands**
RL: PEP (Physical, engineering or chemical process); PYP (Physical process); PROC (Process)
(Affinity; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Analysis
(Binding; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Analysis

(Cell-based reporter; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Samples
(Natural; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Extraction
(**Size-exclusion**-based; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Analysis
(biochem.; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Immunoassay
(enzyme-linked immunosorbent assay; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Liquid chromatographic columns
(gel filtration or **size exclusion**, high-performance; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Mass spectrometry
(liq. chromatog. combined with; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT **Liquid chromatography**
(mass spectrometry combined with; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Solvents
(org.; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Acids, uses
RL: NUU (Other use, unclassified); USES (Uses)
(org.; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT Affinity
Concentration (condition)
Dissociation
Drug screening
IR spectroscopy
Liquid chromatography
Mass spectrometry
Mixing
Mixtures
Molecular weight
Molecules
NMR spectroscopy
Reaction
Solutions
Standard substances, analytical
Ultrafilters
Ultrafiltration
(**size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT **Proteins**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**size-exclusion**-based extn. of affinity ligands and

active compds. from natural samples)
IT Separation
(**size-exclusion; size-exclusion**
-based extn. of affinity ligands and active compds. from natural samples)
IT 9001-03-0
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(II; **size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)
IT 59-66-5, Acetazolamide
RL: ARG (Analytical reagent use); PEP (Physical, engineering or chemical process); PYP (Physical process); ANST (Analytical study); PROC (Process); USES (Uses)
(**size-exclusion**-based extn. of affinity ligands and active compds. from natural samples)

L59 ANSWER 3 OF 10 WPIX (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 2003-112176 [10] WPIX
DOC. NO. NON-CPI: N2003-089272
DOC. NO. CPI: C2003-028786
TITLE: Determining the binding properties of **ligands**, useful for **screening** combinatorial libraries and determining **ligand** affinity, based on competitive reaction.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): BERTLING, W M; HOEFNER, G; KOSAK, H; WANNER, K; BERTLING, W
PATENT ASSIGNEE(S): (NOVE-N) NOVEMBER GES MOLEKULARE MED AG
COUNTRY COUNT: 100
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002095403	A2	20021128	(200310)*	GE	44

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW

DE 10125258	A1	20030109	(200312)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002095403	A2	WO 2002-EP5543	20020521
DE 10125258	A1	DE 2001-10125258	20010523

PRIORITY APPLN. INFO: DE 2001-10125258 20010523
AB WO 200295403 A UPAB: 20030211
NOVELTY - Method for determining the binding properties of a **ligand** (I) that binds specifically to at least one binding site on a target molecule (II).

DETAILED DESCRIPTION - Method for determining the binding properties of a **ligand** (I) that binds specifically to at least one binding site on a target molecule (II) comprises:

(a) first preparing:

(i) a mixture (A) of (I), concentration K1; (II), concentration K3, and a marker (III), concentration K2, that also binds specifically to (II); and

(ii) a mixture (B) of (II) and (III), then incubating the mixtures under identical conditions that allow binding of (I) and (III) to (II)

(b) bound markers (GM1, GM2) are separated from (A) and (B) and the concentrations (K4, K5) of unbound (III) in (A) and (B) determined;

(c) from K5, a concentration K6, that corresponds to concentration of unbound marker in (B) under the assumption that, in (B), concentrations K2 and K3 have been contacted, is determined, then the binding properties of (I) determined from the ratio of K6 to K4.

Alternatively, the amounts (M1, M2) of bound markers are determined, an amount (M3) determined for (B), using the same assumption as above, and the **ligand** properties are determined from the ratio of M3 to M1.
The marker is present in native form and the determination of K4/K5, or M1/M2, is by mass spectrometry.

USE - The method is particularly used to **screen** combinatorial chemical libraries and to determine the affinity of (I).

ADVANTAGE - The method allows relatively simple detection of binding properties of any **ligand** on any target and is not affected by the kinetics of the binding reaction. Target molecules are in native form (so results are physiologically relevant), and the method does not require quantification of the **ligand** itself (often difficult) but rather of the marker. Mass spectrometry allows simultaneous determination of many different markers; even **ligands** of very low affinity can be analyzed and non-specific binding of **ligands** has no significant effect on the results.
Dwg.0/2

L59 ANSWER 4 OF 10 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

ACCESSION NUMBER:	2002249477 EMBASE
TITLE:	High throughput in drug discovery.
AUTHOR:	Kubinyi H.
CORPORATE SOURCE:	H. Kubinyi, University of Heidelberg, BASF AG, Ludwigshafen, Germany. kubinyi@t-online.de
SOURCE:	Drug Discovery Today, (1 Jul 2002) 7/13 (707-709). ISSN: 1359-6446 CODEN: DDTOFS
PUBLISHER IDENT.:	S 1359-6446(02)02323-1
COUNTRY:	United Kingdom
DOCUMENT TYPE:	Journal; (Short Survey)
FILE SEGMENT:	036 Health Policy, Economics and Management 037 Drug Literature Index
LANGUAGE:	English

CT Medical Descriptors:

*drug research
drug cost
drug industry
validation process
productivity
DNA microarray
DNA library
gene expression
drug screening

cell assay
molecular cloning
protein expression
chromatophore
laser
knockout gene
high performance liquid chromatography
proteomics
ligand binding
fluorescence
deletion mutant
enzyme activity
binding affinity
binding site
hydrogen bond
X ray crystallography
protein tertiary structure
drug metabolism
drug design
chemical genetics
ion channel
amino acid sequence
human
short survey
Drug Descriptors:
*new drug: DV, drug development
*new drug: PE, pharmacoeconomics
complementary DNA
aequorin
G protein coupled receptor
ligand
calcium ion
beta galactosidase
phosphotransferase
cytochrome P450
isoenzyme
oligonucleotide
peptide
protein
metalloproteinase inhibitor
cysteine
nucleic acid
protein inhibitor
RN (aequorin) 50934-79-7; (calcium ion) 14127-61-8; (phosphotransferase) 9031-09-8, 9031-44-1; (cytochrome P450) 9035-51-2; (protein) 67254-75-5; (cysteine) 4371-52-2, 52-89-1, 52-90-4

L59 ANSWER 5 OF 10 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:904728 HCAPLUS
DOCUMENT NUMBER: 136:17718
TITLE: Method for determining the quantity of ligands that are bound to target molecules
INVENTOR(S): Wanner, Klaus; Hofner, Georg; Bertling, Wolf
PATENT ASSIGNEE(S): November Aktiengesellschaft Gesellschaft Fur Molekulare Medizin, Germany
SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001094943	A2	20011213	WO 2001-DE2086	20010606
WO 2001094943	A3	20020418		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

DE 10028186	A1	20020919	DE 2000-10028186	20000609

PRIORITY APPLN. INFO.: DE 2000-10028186 A 20000609

AB The invention provides a method for detg. the quantity of ligands that are bound to target mols. The method comprises (a) prepg. the target mols. and a mixed phase contg. a predetd. quantity of ligands in native form; (b) bringing the mixed phase into contact with the target mols. and incubating said phase; (c) sepg. bound ligands from the mixed phase under conditions, in which the quantity of unbound ligands remains const.; (d) detg. the quantity of unbound ligands in the mixed phase; and (e) detg. the quantity of bound ligands, by calcg. the difference between the quantity of ligands as per step (a) and the quantity of ligands detd. as per step (d), whereby the mixed phase as per step (a) contains different ligands which act in part as a ref.

IC ICM G01N033-53
CC 9-16 (Biochemical Methods)
IT Adsorption
Affinity chromatography
Animal tissue
Capillary electrophoresis
Centrifugation
Dialysis
Drug screening
Electrochemical analysis
Filtration
Fluorometry
Gas chromatography
HPLC
Immobilization, molecular
Liposomes
Liquid chromatography
Luminescence spectroscopy
Mass spectrometry
Membranes, nonbiological
Molecular association
Precipitation (chemical)
Reversed phase liquid chromatography
Separation
Spectrophotometry
UV and visible spectroscopy

Ultrafiltration
Virus
(method for detg. quantity of ligands bound to target mols.)
IT Antibodies
Carbohydrates, biological studies
Enzymes, biological studies
Hormones, animal, biological studies
Ion channel
Ligands
Natural products
Nucleic acids
Peptides, biological studies
Polymers, biological studies
Proteins
Receptors
Transport proteins
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(method for detg. quantity of ligands bound to target mols.)

L59 ANSWER 6 OF 10 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:14225 HCAPLUS
DOCUMENT NUMBER: 134:202390
TITLE: MS/NMR: A Structure-Based Approach for Discovering Protein Ligands and for Drug Design by Coupling **Size Exclusion** Chromatography, Mass Spectrometry, and Nuclear Magnetic Resonance Spectroscopy
AUTHOR(S): Moy, Franklin J.; Haraki, Kevin; Mobilio, Dominick; Walker, Gary; Powers, Robert; Tabei, Keiko; Tong, Hui; Siegel, Marshall M.
CORPORATE SOURCE: Department of Biological Chemistry, Wyeth Research, Cambridge, MA, 02140, USA
SOURCE: Analytical Chemistry (2001), 73(3), 571-581
CODEN: ANCHAM; ISSN: 0003-2700
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A protocol is described for rapidly screening small org. mols. for their ability to bind a target protein while obtaining structure-related information as part of a structure-based drug discovery and design program. The methodol. takes advantage of and combines the inherent strengths of **size exclusion** gel chromatog., mass spectrometry, and NMR to identify bound complexes in a relatively universal high-throughput screening approach. **Size exclusion** gel chromatog. in the spin column format provides the high-speed sepn. of a protein-ligand complex from free ligands. The spin column eluent is then analyzed under denaturing conditions by electrospray ionization mass spectrometry (MS) for the presence of small mol. wt. compds. formerly bound to the protein. Hits identified by MS are then individually assayed by chem. shift perturbations in a 2D 1H-15N HSQC NMR spectrum to verify specific interactions of the compd. with the protein and identification of the binding site on the protein. The utility of the MS/NMR assay is demonstrated with the use of the catalytic fragment of human fibroblast collagenase (MMP-1) as a target protein and the screening of a library consisting of .apprx.32 000 compds. for the identification of mols. that exhibit specific binding to the RGS4 protein.
CC 1-1 (Pharmacology)

ST drug design protein ligand MS NMR; mass spectrometry drug design protein ligand; NMR drug design protein ligand; coupling **size exclusion** chromatog drug design

IT Combinatorial library
Drug design
Drug screening
Electrospray ionization mass spectrometry
Molecular association
NMR spectroscopy
Size-exclusion chromatography
(MS/NMR as structure-based approach for discovering protein ligands and for drug design by coupling **size exclusion** chromatog. and mass spectrometry and NMR spectroscopy applied to library of MMP-1 inhibitors)

IT **Ligands**
Proteins, general, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(MS/NMR as structure-based approach for discovering protein ligands and for drug design by coupling **size exclusion** chromatog. and mass spectrometry and NMR spectroscopy applied to library of MMP-1 inhibitors)

IT 161314-70-1 206258-35-7 206547-13-9 206550-47-2 212766-11-5
212766-47-7 212766-65-9 233754-03-5 239796-71-5 239796-72-6
328408-71-5 328408-72-6 328408-73-7
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(MS/NMR as structure-based approach for discovering protein ligands and for drug design by coupling **size exclusion** chromatog. and mass spectrometry and NMR spectroscopy applied to library of MMP-1 inhibitors)

IT 9001-12-1, Matrix metalloproteinase 1
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(MS/NMR as structure-based approach for discovering protein ligands and for drug design by coupling **size exclusion** chromatog. and mass spectrometry and NMR spectroscopy applied to library of MMP-1 inhibitors)

REFERENCE COUNT: 80 THERE ARE 80 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L59 ANSWER 7 OF 10 MEDLINE
ACCESSION NUMBER: 2002063997 MEDLINE
DOCUMENT NUMBER: 21647146 PubMed ID: 11789692
TITLE: Biological libraries.
AUTHOR: Dani M
CORPORATE SOURCE: TECNOGEN SCpA, Piana di Monte Verna (CE), Italy.
SOURCE: JOURNAL OF RECEPTOR AND SIGNAL TRANSDUCTION RESEARCH, (2001 Nov) 21 (4) 447-68. Ref: 107
Journal code: 9509432. ISSN: 1079-9893.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020710
Entered Medline: 20020709
CT Check Tags: Animal
Bacteria: GE, genetics
Bacteriophage lambda: GE, genetics
Chromatography, Affinity
DNA, Complementary: GE, genetics
Drug Design
Drug Evaluation, Preclinical
Ligands
*Peptide Library
Proteins: GE, genetics
Saccharomyces cerevisiae: GE, genetics
CN 0 (DNA, Complementary); 0 (Ligands); 0 (Peptide Library); 0 (Proteins)

L59 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2000:574020 HCAPLUS
DOCUMENT NUMBER: 133:159918
TITLE: High throughput **size-exclusive** method of screening complex biological materials for affinity ligands
INVENTOR(S): Dunayevskiy, Yuriy M.; Hughes, Dallas E.
PATENT ASSIGNEE(S): Cetek Corporation, USA
SOURCE: PCT Int. Appl., 33 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000047999	A1	20000817	WO 2000-US3562	20000211
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1151301	A1	20011107	EP 2000-908605	20000211
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002541435	T2	20021203	JP 2000-598857	20000211
US 2002052006	A1	20020502	US 2001-920435	20010801

PRIORITY APPLN. INFO.: US 1999-119966P P 19990212
WO 2000-US3562 W 20000211

AB The invention encompasses an improved, rapid, **size-exclusive** method for screening complex biol. materials, e.g., combinatorial libraries, natural products or samples, or mixts. of pure compds., for small mol. wt. ligands that bind specifically to a protein target, using **size-exclusion** sepn., ultrafiltration, and mass spectrometry.
IC ICM G01N033-537
CC 1-1 (Pharmacology)
Section cross-reference(s): 9
IT Biological materials
Combinatorial library
Drug screening

HPLC
Mass spectrometry
Molecular association
Separation
Ultrafilters
Ultrafiltration
(high throughput **size-exclusive** method of screening complex biol. materials for affinity ligands using **size-exclusion** sepn. and ultrafiltration and mass spectrometry)

IT **Ligands**
Natural products, pharmaceutical
Proteins, general, biological studies
Receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(high throughput **size-exclusive** method of screening complex biol. materials for affinity ligands using **size-exclusion** sepn. and ultrafiltration and mass spectrometry)

IT Mass spectrometry
Mass spectrometry
(liq. chromatog. combined with; high throughput **size-exclusive** method of screening complex biol. materials for affinity ligands using **size-exclusion** sepn. and ultrafiltration and mass spectrometry)

IT **Liquid chromatography**
Liquid chromatography
(mass spectrometry combined with; high throughput **size-exclusive** method of screening complex biol. materials for affinity ligands using **size-exclusion** sepn. and ultrafiltration and mass spectrometry)

IT 9001-03-0
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(II, inhibitors, screening of; high throughput **size-exclusive** method of screening complex biol. materials for affinity ligands using **size-exclusion** sepn. and ultrafiltration and mass spectrometry)

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L59 ANSWER 9 OF 10 MEDLINE
ACCESSION NUMBER: 97389454 MEDLINE
DOCUMENT NUMBER: 97389454 PubMed ID: 9246636
TITLE: Affinity selection and mass spectrometry-based strategies to identify lead compounds in combinatorial libraries.
AUTHOR: Kaur S; McGuire L; Tang D; Dollinger G; Huebner V
CORPORATE SOURCE: Chiron Corp., Emeryville, California 94608-2916, USA.
SOURCE: JOURNAL OF PROTEIN CHEMISTRY, (1997 Jul) 16 (5) 505-11.
Journal code: 8217321. ISSN: 0277-8033.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970922
Last Updated on STN: 19970922
Entered Medline: 19970911
AB The screening of diverse libraries of small molecules created by

combinatorial synthetic methods is a recent development which has the potential to accelerate the identification of lead compounds in drug discovery. We have developed a direct and rapid method to identify lead compounds in libraries involving affinity selection and mass spectrometry. In our strategy, the receptor or target molecule of interest is used to isolate the active components from the library physically, followed by direct structural identification of the active compounds bound to the target molecule by mass spectrometry. In a drug design strategy, structurally diverse libraries can be used for the initial identification of lead compounds. Once lead compounds have been identified, libraries containing compounds chemically similar to the lead compound can be generated and used to optimize the binding characteristics. These strategies have also been adopted for more detailed studies of protein-ligand interactions.

CT Binding, Competitive
***Chromatography, Affinity: MT, methods**
Drug Design
Drug Evaluation, Preclinical: MT, methods
Ligands
*Peptide Library
***Proteins: AN, analysis**
Proteins: ME, metabolism
Receptors, Drug: ME, metabolism
*Spectrum Analysis, Mass: MT, methods
Structure-Activity Relationship

CN 0 (Ligands); 0 (Peptide Library); 0 (Proteins); 0 (Receptors, Drug)

L59 ANSWER 10 OF 10 WPIX (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 1990-123499 [16] WPIX
CROSS REFERENCE: 1988-235072 [33]; 1990-067084 [09]
DOC. NO. CPI: C1990-054310
TITLE: Polymer matrix with surface hydrophilicity - produced from nitrile-contg. polymer modified to form substd. amide gps. on surface.
DERWENT CLASS: A14 A88 B04 F01 J01
INVENTOR(S): HODGINS, L T; SAMUELSON, E
PATENT ASSIGNEE(S): (MEMB-N) MEMBREX INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 4906379	A	19900306	(199016)*		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 4906379	A	US 1988-149552	19880128

PRIORITY APPLN. INFO: US 1987-7623 19870128; US 1988-149552 19880128
AB US 4906379 A UPAB: 19950306
Matrix comprises molecules of a nitrile-contg. polymer (I) which provides solely on the surface of the matrix sufficient unchanged, substd. amide gps. to render the surface hydrophilic, or which provides uncharged,

hydrophilic polar gps. obtd. by derivatisation of reactive pendent gps. to impart hydrophilicity. **Ligands** attached to the surface, pref. comprising a bio-selective affinity gp., esp. a nucleic acid, polynucleotide, monosaccharide, polysaccharide, lipid, amino acid, peptide, **protein**, hormone, vitamin, metabolic cofactor, drug or antibiotic.

The substd. amide gps. are derived from nitrile gps. of the nitrile-contg. polymer, or are grafted to the polymer or attached to monomers which are grafted to the polymer. The polymer comprises an acrylonitrile-type monomer, esp. (meth)acrylonitrile. The amide gps. are N-methylolamide gps. The polymer may be crosslinked by means of a methylene-bis-amide.

USE/ADVANTAGE - Used for prepn. of filters, membranes, beads, non-spherical particles, hollow fibres, solid fibres, rods, fabrics, **screens** or sepn. media, for (**ultra**)**filtration**, reverse osmosis, dialysis, pervaporation, sieving, affinity **chromatography**, affinity purification, etc. Surface modification provides articles having superior physical integrity to withstand pressure driven sepns. and hydrophilic surfaces to prevent fouling. @(10pp Dwg.No.0/1)
0/1

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=> d que
L64          85 SEA (ALIS AND (NEOGENESIS OR NEO GENESIS)) OR AUTOMAT? LIGAND
                IDENT? SYSTEM
L66           9 SEA L64 AND P/DT
L67          76 SEA L64 NOT L66
L68          68 DUP REM L67 (8 DUPLICATES REMOVED)
L69           2 SEA L68 NOT PY>2000
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2 oldest non-Patent Refs to ALIS

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=> d ibib ab hitind 1-2

L69  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:        2000:671517  CAPLUS
DOCUMENT NUMBER:         134:276228
TITLE:                   The keys to chemical genomics
AUTHOR(S):               Pal, Kollol
CORPORATE SOURCE:        Neo Genesis Drug Discovery, Inc., Cambridge, MA, USA
SOURCE:                  Modern Drug Discovery (2000), 3(7), 47,49-50,53,55
                         CODEN: MDDIFT; ISSN: 1099-8209
PUBLISHER:               American Chemical Society
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   The NeoGenesis approach to chem. genomics-called ALIS
     (automated ligand identification
     system)-is presented.  Affinity selection, involves the binding of
     ligands to a target, is a powerful yet elegant strategy for screening
     numerous genomic targets.  This ultrahigh-throughput screening technol.
     has three distinct components: incubation of ligand pools with a target
     protein, sepn. of the protein ligand complex from unbound ligands, and
     mass spectral detection of dissocd. ligands.
CC   3-1 (Biochemical Genetics)
     Section cross-reference(s): 6
ST   chem genomics automated ligand identification
     system; drug discovery therapeutic algorithm target protein
IT   Ligands
     RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (ALIS screening technol. of; keys to chem. genomics)
REFERENCE COUNT:         6     THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS
                               RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L69  ANSWER 2 OF 2  CEABA-VTB  COPYRIGHT  2003 DECHEMA
ACCESSION NUMBER:        2001(03):5111  CEABA-VTB   FILE SEGMENT   B
TITLE:                   The keys to chemical genomics
AUTHOR:                  Pal, K.
CORPORATE SOURCE:        NeoGenesis Drug Discovery, Inc., Cambridge MA, USA
SOURCE:                  mdd, modern drug discovery (2000) 3(7), 6
                         Reference(s), 46-47,49-50,53,55, 3f
                         ISSN: 1099-8209
LANGUAGE:                English
SUMMARY LANGUAGE:        English
AB   A presentation of the ALIS (automated ligand
     identification system) developed by NeoGenesis
     for screening large numbers of protein targets, based on the concept of
     chemical genomics, whereby the affinity of a small molecule for a target
     protein is used as a first measure of the drug potential of that
     molecule. This technology has three components, namely, incubation of
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ligand pools with a target protein, separation of the protein-ligand complex from unbound ligands using micro-scale size-exclusion chromatography, and MS detection of dissociated ligands. Currently, 300,000 compounds can be processed daily.(MacMillan, Duncan)

Patent Refs. to ALIS

L66 ANSWER 1 OF 9 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 2002080955 PCTFULL ED 20021028 EW 200242
TITLE (ENGLISH): STIMULATION OF OSTEOGENESIS USING RANK LIGAND FUSION PROTEINS
TITLE (FRENCH): STIMULATION D'OSTEOGENESE UTILISANT DES PROTEINES DE FUSION DE LIGANDS RANK
INVENTOR(S): LAM, Jonathan, Barnes-Jewish Hospital, 216 South Kingshighway, P.O. Box 14109, St. Louis, MO 63178, US; ROSS, F., Patrick, Barnes-Jewish Hospital, 216 South Kingshighway, P.O. Box 14109, St. Louis, MO 63178, US; TEITELBAUM, Steven, L., Barnes-Jewish Hospital, 216 South Kingshighway, P.O. Box 14109, St. Louis, MO 63178, US
PATENT ASSIGNEE(S): BARNES-JEWISH HOSPITAL, 216 South Kingshighway, P.O. Box 14109, St. Louis, MO 63178, US [US, US]
AGENT: BLOSSER, G., Harley$, Senniger, Powers, Leavitt & Roedel, 16th floor, One Metropolitan Square, St. Louis, MO 63102$, US
LANGUAGE OF FILING: English
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 2002080955	A1	20021017

DESIGNATED STATES
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
RW (ARIPO): GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
RW (EAPO): AM AZ BY KG KZ MD RU TJ TM
RW (EPO): AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
RW (OAPI): BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
APPLICATION INFO.: WO 2002-US9271 A 20020322
PRIORITY INFO.: US 2001-60/277,855 20010322
US 2001-60/311,163 20010809
US 2001-60/328,876 20011012
US 2001-60/329,231 20011012
US 2001-60/329,393 20011015

ABEN A method of enhancing bone formation comprising administering an effective amount of 1) an oligomeric complex of one or more of RANKL, a RANKL fusion protein or an analog, derivative or mimic thereof, 2) an osteogenic compound capable of enhancing activity of one or more intracellular proteins in osteoblasts or osteoblast precursors, wherein said activity is indicative of bone formation, or 3) an osteogenic compound capable of inactivating one or more phosphatases in osteoblasts or osteoblast precursors, wherein said inactivation is indicative of bone formation. The method also may be used to treat a disease or condition manifested at least in part by the loss of bone mass by administering to a patient a pharmaceutical composition comprising an oligomeric complex or osteogenic compound disclosed herein.
ABFR L'invention concerne un procede d'amelioration de la formation osseuse, consistant a administrer une quantite efficace 1) d'un complexe

oligomerique d'un ou plusieurs elements parmi RANKL, une proteine de fusion RANKL ou un analogue, un derive ou un mimetique de cette derniere, 2) d'un compose osteogenique capable d'ameliorer l'activite d'une ou de plusieurs proteines intracellulaires dans des osteoblastes ou des precurseurs d'osteoblastes, ladite activite etant indicative d'une formation osseuse, ou 3) d'un compose osteogenique capable d'inactiver une ou plusieurs phosphatases dans des osteoblastes ou des precurseurs d'osteoblastes, ladite inactivation etant indicative d'une formation osseuse. Le procede peut egalement etre utilise pour traiter une maladie ou une condition se manifestant au moins en partie par la perte de masse osseuse, en administrant a un patient une composition pharmaceutique renfermant un complexe oligomerique ou un compose osteogenique decrit ci-dessus.

L66 ANSWER 2 OF 9 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 2002057792 PCTFULL ED 20020801 EW 200230
TITLE (ENGLISH): AFFINITY SELECTION-BASED SCREENING OF HYDROPHOBIC PROTEINS
TITLE (FRENCH): CRIBLAGE DE PROTEINES HYDROPHOBES PAR SELECTION D'AFFINITES
INVENTOR(S): FELSCH, Jason, S., 11 Chase Road, Waltham, MA 02452-6401, US;
ANNIS, David, Allen, Jr., 14 Remington Street, Cambridge, MA 02138, US;
KALGHATGI, Krishna, 25 Jacob Amsden Road, Westboro, MA 01581, US;
NASH, Huw, M., 109 River Street 3-B, Cambridge, MA 02139, US
PATENT ASSIGNEE(S): NEOGENESIS PHARMACEUTICALS, INC., 840 Memorial Drive, Cambridge, MA 02139, US [US, US]
AGENT: MCISAAC, Robert$, Hale and Dorr LLP, 60 State Street, Boston, MA 02109$, US
LANGUAGE OF FILING: English
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 2002057792	A2	20020725

DESIGNATED STATES
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
RW (ARIPO): GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
RW (EAPO): AM AZ BY KG KZ MD RU TJ TM
RW (EPO): AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
RW (OAPI): BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
APPLICATION INFO.: WO 2001-US50088 A 20011219
PRIORITY INFO.: US 2000-60/258,970 20001229
ABEN The invention relates to methods based on affinity selection for the identification
of ligands for hydrophobic proteins bound by amphiphile. The invention also
provides hydrophobic proteins and methods of isolation of hydrophobic

proteins
that are suitable for ligand screening.
ABFR L'invention porte sur des procedes se basant sur la selection d'affinites pour identifier les ligands de proteines hydrophobes liees par un amphiphile; et sur des proteines hydrophobes et des procedes
d'isolement de proteines hydrophobes adaptees au criblage des ligands.

L66 ANSWER 3 OF 9 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 2002020436 PCTFULL ED 20020705 EW 200211
TITLE (ENGLISH): METHODS FOR FORMING COMBINATORIAL LIBRARIES COMBINING AMIDE BOND FORMATION WITH EPOXIDE OPENING
TITLE (FRENCH): PROCEDES DE FORMATION DE BIBLIOTHEQUES COMBINATOIRES COMBINANT LA FORMATION DE LIAISONS AMIDE ET L'OUVERTURE D'EPOXYDE
INVENTOR(S): SHIPPS, Gerald, W., 76 Pleasant Street, Stoneham, MA 02180, US;
ROSNER, Kristin, E., 395 Broadway #R2A, Cambridge, MA 02139, US;
MAKARA, Gergely, M., 175-F Centre Street, #608, Quincy, MA 02169, US;
WINTNER, Edward, A., 44 Valentine Street, Cambridge, MA 02139, US;
NASH, Huw, M., 109 River Street 3-B, Cambridge, MA 02139, US;
FELSCH, Jason, S., 11 Chase Road, Waltham, MA 02452, US;
PAL, Kollol, 205 Tudor Road, Needham, MA 02492, US;
LENZ, George, R., 6 Apple Blossom Road, Andover, MA 01810, US
PATENT ASSIGNEE(S): NEOGENESIS PHARMACEUTICALS, INC., 840 Memorial Drive, Cambridge, MA 02139, US [US, US]
AGENT: KLUNDER, Janice, M.$, Hale and Dorr LLP, 60 State Street, Boston, MA 02109$, US
LANGUAGE OF FILING: English
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 2002020436	A2	20020314

DESIGNATED STATES
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
RW (ARIPO): GH GM KE LS MW MZ SD SL SZ TZ UG ZW
RW (EAPO): AM AZ BY KG KZ MD RU TJ TM
RW (EPO): AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
RW (OAPI): BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
APPLICATION INFO.: WO 2001-US27226 A 20010831
PRIORITY INFO.: US 2000-60/230,122 20000905
ABEN The invention relates to methods for forming combinatorial libraries. The invention provides methods suitable for the rapid and convenient

synthesis of very large combinatorial libraries of small organic molecules. In particular, the invention provides a method for forming combinatorial libraries combining amide bond formation with epoxide opening.

ABFR L'invention concerne des procedes de formation de bibliotheques combinatoires. L'invention concerne des procedes indiques pour la synthese rapide et facile de tres grandes bibliotheques combinatoires de petites molecules organiques. L'invention concerne notamment un procede permettant de former des bibliotheques combinatoires, combinant la formation de liaisons amide et l'ouverture d'epoxyde.

L66 ANSWER 4 OF 9 USPATFULL
ACCESSION NUMBER: 2003:17899 USPATFULL
TITLE: Stimulation of osteogenesis using rank ligand fusion proteins
INVENTOR(S): Lam, Jonathan, West Memphis, AR, UNITED STATES
Ross, F. Patrick, Olivette, MO, UNITED STATES
Teitelbaum, Steven L., University City, MO, UNITED STATES
PATENT ASSIGNEE(S): Barnes-Jewish Hospital (2)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003013651	A1	20030116	
APPLICATION INFO.:	US 2002-105057	A1	20020322	(10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-277855P	20010322 (60)
	US 2001-311163P	20010809 (60)
	US 2001-329231P	20011012 (60)
	US 2001-328876P	20011012 (60)
	US 2001-329393P	20011015 (60)

DOCUMENT TYPE: **Utility**
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SONNENSCHEIN NATH & ROSENTHAL, P.O. BOX 061080, WACKER DRIVE STATION, CHICAGO, IL, 60606-1080
NUMBER OF CLAIMS: 38
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 18 Drawing Page(s)
LINE COUNT: 1942
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of enhancing bone formation comprising administering an effective amount of 1) an oligomeric complex of one or more of RANKL, a RANKL fusion protein or an analog, derivative or mimic thereof, 2) an osteogenic compound capable of enhancing activity of one or more intracellular proteins in osteoblasts or osteoblast precursors, wherein said activity is indicative of bone formation, or 3) an osteogenic compound capable of inactivating one or more phosphatases in osteoblasts or osteoblast precursors, wherein said inactivation is indicative of bone formation. The method also may be used to treat a disease or condition manifested at least in part by the loss of bone mass by administering to a patient a pharmaceutical composition comprising an oligomeric complex or osteogenic compound disclosed herein.

L66 ANSWER 5 OF 9 USPATFULL
ACCESSION NUMBER: 2002:294574 USPATFULL

TITLE: Affinity selection-based screening of hydrophobic proteins
INVENTOR(S): Felsch, Jason S., Waltham, MA, UNITED STATES
Annis, David Allen, JR., Cambridge, MA, UNITED STATES
Kalghatgi, Krishna, Westboro, MA, UNITED STATES
Nash, Huw M., Cambridge, MA, UNITED STATES
PATENT ASSIGNEE(S): NeoGenesis Pharmaceuticals, Inc., Cambridge, MA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002164617	A1	20021107
APPLICATION INFO.:	US 2001-29009	A1	20011219 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-258970P	20001229 (60)

DOCUMENT TYPE: **Utility**
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HALE AND DORR, LLP, 60 STATE STREET, BOSTON, MA, 02109
NUMBER OF CLAIMS: 40
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 10 Drawing Page(s)
LINE COUNT: 2501
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to methods based on affinity selection for the identification of ligands for hydrophobic proteins bound by amphiphile. The invention also provides hydrophobic proteins and methods of isolation of hydrophobic proteins that are suitable for ligand screening.

L66 ANSWER 6 OF 9 USPATFULL
ACCESSION NUMBER: 2002:149331 USPATFULL
TITLE: Methods for forming combinatorial libraries combining amide bond formation with epoxide opening
INVENTOR(S): Shipps, Gerald W., Stoneham, MA, UNITED STATES
Rosner, Kristin E., Cambridge, MA, UNITED STATES
Makara, Gergely M., Quincy, MA, UNITED STATES
Wintner, Edward A., Cambridge, MA, UNITED STATES
Nash, Huw M., Cambridge, MA, UNITED STATES
Felsch, Jason S., Waltham, MA, UNITED STATES
Pal, Kollol, Needham, MA, UNITED STATES
Lenz, George R., Andover, MA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002077491	A1	20020620
APPLICATION INFO.:	US 2001-943852	A1	20010831 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-230122P	20000905 (60)

DOCUMENT TYPE: **Utility**
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Janice M. Klunder, Hale and Dorr, 60 State Street, Boston, MA, 02109
NUMBER OF CLAIMS: 44

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Page(s)
LINE COUNT: 2245
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to methods for forming combinatorial libraries. The invention provides methods suitable for the rapid and convenient synthesis of very large combinatorial libraries of small organic molecules. In particular, the invention provides a method for forming combinatorial libraries combining amide bond formation with epoxide opening.

L66 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:185035 CAPLUS
DOCUMENT NUMBER: 136:247586
TITLE: Methods for forming combinatorial libraries combining amide bond formation with epoxide opening
INVENTOR(S): Shipps, Gerald W.; Rosner, Kristin E.; Makara, Gergely M.; Wintner, Edward A.; Nash, Huw M.; Felsch, Jason S.; Pal, Kollol; Lenz, George R.
PATENT ASSIGNEE(S): Neogenesis Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 139 pp.
CODEN: PIXXD2
DOCUMENT TYPE: **Patent**
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002020436	A2	20020314	WO 2001-US27226	20010831
WO 2002020436	A3	20030109		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001088617	A5	20020322	AU 2001-88617	20010831
US 2002077491	A1	20020620	US 2001-943852	20010831

PRIORITY APPLN. INFO.: US 2000-230122P P 20000905
WO 2001-US27226 W 20010831
OTHER SOURCE(S): CASREACT 136:247586; MARPAT 136:247586
AB The invention relates to methods for forming combinatorial libraries. The invention provides methods suitable for the rapid and convenient synthesis of very large combinatorial libraries of small org. mols. In particular, the invention provides a method for forming combinatorial libraries combining amide bond formation with epoxide opening. A method of forming a combinatorial library of compds. comprises reacting a plurality of core mols. (epoxides) with a mixt. of nucleophilic building blocks (amines) in a reaction vessel to form a library of compds., wherein each of said core mols. comprises (i) an acid halide, sulfonyl halide, isocyanate or isocyanate equiv., or activated ester functional group; and (ii) an epoxide functional group. More specifically, the method comprises sequentially (i) contacting the core mols. (epoxides) with a mixt. of

amine building blocks so that reaction with the acid halide or activated ester functional groups is achieved; and (ii) adding a Lewis acid so that reaction of the amine building blocks with the epoxide functional groups is achieved. A total of 14 core epoxides, e.g. (RS)-, (R)-, or (S)-3-(glycidyloxy)isoxazole-5-carboxylic acid pentafluorophenyl ester (I), 1-glycidylimidazole-4,5-dicarboxylic acid bis(pentafluorophenyl) ester, 3-(glycidyloxy)-4-methoxybenzoic acid pentafluorophenyl ester, 4-(glycidyloxy)quinoline-2-carboxylic acid pentafluorophenyl ester, lactam (II), and spiroepoxide (III) were prepd. Thus, to a soln. of core epoxide compd. (RS)-I (100 mg, 0.28 mmol) in CH2Cl2/THF (3 mL each) at 24 .degree.C was added a soln. of amine building blocks (0.019 mmol each), e.g. N-(2-chlorophenyl)piperazine and (R)-1-(4-methoxyphenyl)ethylamine, as a soln. in CH2Cl2/THF (3 mL each) and an Yb(OTf)3 catalyst soln. (100 .mu.L of a 120 mg soln. in 1.5 mL THF) and DIEA (50 .mu.L, 0.28 mmol) were added. The mixt. was heated to 45-50.degree. for 24 h, then cooled, treated with Amberlite (100 mg), stirred for an addnl. 1 h at 24.degree., and then filtered and concd. to yield the soln.-phase library as a slightly yellow film contg. 512 substitutionally and stereochem. unique compds., e.g. (IV) and (V). The library was screened by **automated ligand identification system** (ALIS) screening of E. coli dihydrofolate reductase (DHFRec).

IC ICM C07B061-00

CC 28-9 (Heterocyclic Compounds (More Than One Hetero Atom))
Section cross-reference(s): 25, 27

IT 9002-03-3, Dihydrofolate reductase
RL: CUS (Combinatorial use); CMBI (Combinatorial study); USES (Uses)
(**automated ligand identification system** (ALIS) screening of combinatorial libraries combining amide bond formation and epoxide opening of epoxy carboxylic acid pentafluorophenyl ester with amines using E. coli dihydrofolate reductase)

L66 ANSWER 8 OF 9 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01874 BIOTECHDS
TITLE: Identifying ligand for hydrophobic protein based on affinity selection which can operate in the presence of amphiphile without regard to the specific biological function of hydrophobic target protein;
baculo virus vector-mediated FLAG-tagged muscarinic acetylcholine receptor gene transfer and expression in insect cell for drug screening and disease diagnosis
AUTHOR: FELSCH J S; ANNIS D A; KALGHATGI K; NASH H M
PATENT ASSIGNEE: NEOGENESIS PHARM INC
PATENT INFO: WO 2002057792 25 Jul 2002
APPLICATION INFO: WO 2001-US50088 19 Dec 2001
PRIORITY INFO: US 2000-258970 29 Dec 2000; US 2000-258970 29 Dec 2000
DOCUMENT TYPE: **Patent**
LANGUAGE: English
OTHER SOURCE: WPI: 2002-599728 [64]

AB DERWENT ABSTRACT:
NOVELTY - Identifying (M1) a ligand for a hydrophobic protein (HP), comprising selecting a ligand molecule by affinity selection by exposing a hydrophobic target protein bound by an amphiphile to a multiplicity of molecules to promote formation of at least a complex between the hydrophobic target protein and the ligand molecule, separating the complex from the unbound molecules, and identifying the ligand molecule, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) isolating (M2) HP, involves purifying HP by sucrose gradient ultracentrifugation, antibody affinity purification, and immobilized metal affinity chromatography; and (2) an isolated nucleic acid molecule suitable for HP sequence expression, comprising a vector polynucleotide sequence for protein expression in a eukaryotic cell, and a polynucleotide sequence encoding an engineered HP comprising N-terminal methionine residue, heterologous signal sequence (SS), a transmembrane domain sequence, at least two tag sequences useful for affinity selection, and a HP sequence.

BIOTECHNOLOGY - Preferred Method: In (M1), the exposure of the hydrophobic target protein to a multiplicity of molecules occurs under homogenous or heterogenous solution phase conditions. The selection of the ligand molecule is done using multi-dimensional chromatography. The multiplicity of molecules is a mass-coded library of molecules, or a library of molecules that is not mass-coded. The amphiphile is a polar lipid, amphiphilic macromolecular polymer, surfactant or detergent, or amphiphilic polypeptide. The ligand molecule is identified or deconvoluted by mass spectral analysis. The separation of the complex from the unbound molecules is accomplished with solid phase chromatography media. The hydrophobic target protein comprises a transmembrane domain sequence, at least two tag sequences useful for affinity selection, and HP sequence. The tag sequence comprises epitope tag sequences chosen from FLAG tag Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-, EE (NH2-Glu-Glu-Glu-Glu-Tyr-Met-Pro-Met-Glu-COOH), hemagglutinin (NH2-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-COOH), myc tag (NH2-Lys-His-Lys-Leu-Glu-Gln-Leu-Arg-Asn-Ser-Gly-Ala-COOH) and herpes simplex virus (HSV) tag (NH2-Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp-COOH). The hydrophobic target protein comprises a sequence with an amino terminus to carboxy terminus order chosen from Tag1-Tag2-HP, Tag1-HP-Tag2, and HP-Tag1-Tag2. The hydrophobic target protein further comprises a heterologous SS at the amino terminus, such as Mellitin SS of NH2-Lys-Phe-Leu-Val-Asn-Val-Ala-Leu-Val-Phe-Met-Val-Val-Tyr-Ile-Ser-Tyr-Ile-Tyr-Ala-COOH, GP SS of NH2-Val-Arg-Thr-Ala-Val-Leu-Ile-Leu-Leu-Leu-Val-Arg-Phe-Ser-Glu-Pro-COOH, hemagglutinin SS of NH2-Lys-Thr-Ile-Ile-Ala-Leu-Ser-Tyr-Ile-Phe-Cys-Leu-Val-Phe-Ala-COOH, rhodopsin tag 1 SS of 34 amino acids, sequence given in the specification, and rhodopsin tag ID4 SS of NH2-Gly-Lys-Asn-Pro-Leu-Gly-Val-Arg-Lys-Thr-Glu-Thr-Ser-Gln-Val-Ala-Pro-Ala-COOH. The tag sequence further comprises a hexahistidine sequence and a decahistidine sequence. The hydrophobic target protein is GP67 SS-Myc tag-EE tag-human m2 mAChR, Mellitin SS-flag tag-human beta2 adrenergic receptor-EE tag, hemagglutinin SS-human neurokinin 3 receptor-HSV tag-Myc tag, Mellitin SS-flag tag-human m1 mAChR-EE tag, and hemagglutinin SS-rat m3 mAChR-HSV tag-OctaHis tag. Preferred Nucleic Acid: The N-terminal methionine sequence and the heterologous SS is Met-Lys-Phe-Leu-Val-Asn-Val-Ala-Leu-Val-Phe-Met-Val-Val-Tyr-Ile-Ser-Tyr-Ile-Tyr-Ala, Met-Val-Arg-Thr-Ala-Val-Leu-Ile-Leu-Leu-Leu-Val-Arg-Phe-Ser-Glu-Pro, Met-Lys-Thr-Ile-Ile-Ala-Leu-Ser-Tyr-Ile-Phe-Cys-Leu-Val-Phe-Ala, or Met-Gly-Lys-Asn-Pro-Leu-Gly-Val-Arg-Lys-Thr-Glu-Thr-Ser-Gln-Val-Ala-Pro-Ala-COOH.

USE - (M1) is useful for identifying a ligand for HP such as a membrane, integral membrane, transmembrane, monotopic or polytopic membrane, pump, channel, receptor kinase, G protein-coupled receptor, or transporter protein, or membrane-associated enzyme, or Myc tag-EE tag-human m2 mAChR, flag tag-human beta2 adrenergic receptor-EE tag, human neurokinin 3 receptor-HSV tag-Myc tag, flag tag-human m1 mAChR-EE tag, and rat m3 mAChR-HSV tag-OctaHis tag (claimed). The ligand

identified by (M1) is useful for the development of novel medicines and medicinal diagnostics.

EXAMPLE - Identification of ligand binding to m2 mAChR protein by mass spectroscopy was performed as follows: A gene construct encoding the m2 subtype of the muscarinic acetylcholine receptor (m2R) was cloned into a baculovirus expression vector. The gene construct encoded a polypeptide with amino terminal methionine, melittin signal sequence (NH2-KFLVNVALVFMVVYISYIYA-COOH), FLAG M1 epitope tag (NH2-DYKDDDDK-COOH) and m2 muscarinic acetylcholine receptor (NCBI Accession No.X04708). The expression vector was used to generate baculovirus that directed expression of polypeptide in insect cells. To purify FLAG-tagged m2R, 60 g of insect cells expressing the polypeptide were suspended in 0.6 l of TBS (50 mM Tris-HCl, pH 7.4, 100 mM NaCl), and the sample was homogenized and centrifuged. The pellet was discarded, supernatant was ultracentrifuged, and the pelleted cell membranes were resuspended in TBS. The suspension was incubated and ultracentrifuged. The soluble supernatant was applied to a column of FLAT M1 antibody resin for antibody affinity purification. Eluted column fractions containing purified FLAG-tagged m2R were identified. To assess concentration of purified FLAG-tagged m2R protein that was capable of binding muscarinic ligands, glass fiber filter-binding assays were performed. The m2R preparation consisted of 6 micro-M m2R in TBS-D with 100 micro-g/ml of FLAG peptide. Stock cyclooxygenase 1 (COX) and stock discrete ligands pirenzepine, quinuclidinyl bezylate (QNB) and atropine were prepared to 400 micro-M in TBS. Four combinatorial chemical libraries were prepared in dimethyl sulfoxide (DMSO), which were designated NMG-66, NGM-41, NGL-10-A-41 and NGL-116-A-470, respectively. Four stock test libraries were prepared containing atropine and QNB. Binding reactions were prepared that combined protein (either m2R test protein or COX control protein) with ligand or protein with DMSO as a control. In each case, 38 micro-l of premix buffer was dispensed into tubes containing 2 micro-l of DMSO or DMSO-solubilized ligands, mixed by vortexing, and centrifuged. The supernatants were transferred to tubes at 4 degrees C. Target protein m2R or control protein COX was added to the supernatants, mixed well, and incubated. These binding reaction preparations were then subjected to **automated ligand identification system** (ALIS) analysis. Ligands that bound to the m2R with suitably high affinity were collected with the protein-containing size exclusion chromatography (SEC) fraction. The large detergent-solubilized molecules were separated from the unbound small drug-like molecules by SEC. The protein containing fraction was identified with ultraviolet electronic absorption spectrometry monitoring at 230 nm, and transferred by a sample loop to a low flowrate reverse phase chromatography (RPC) system. RPC column was maintained at 60 degrees C to promote ligand dissociation from the complex. From the column, the ligand was eluted into a high-resolution mass spectrometer for analysis using a gradient of 5-95 % acetonitrile in water over 5 minutes. Mass analyzed ligands collected with protein-containing SEC fraction by virtue of its high affinity for the protein-detergent m2R complex were identified by fore-knowledge of their precise mass. Experiments demonstrated that m2R screened by ALIS analysis enabled known m2R ligands to be extracted from mixtures of a multiplicity of small drug like molecules by virtue of the known m2R ligands high affinity for m2R detergent complex. This ALIS-formatted screen recovered m2R ligands from drug libraries and ligands bound to the m2R-detergent complex in the absence of drug libraries.(97 pages)

L66 ANSWER 9 OF 9 CASREACT COPYRIGHT 2003 ACS
ACCESSION NUMBER: 136:247586 CASREACT
TITLE: Methods for forming combinatorial libraries combining amide bond formation with epoxide opening
INVENTOR(S): Shipps, Gerald W.; Rosner, Kristin E.; Makara, Gergely M.; Wintner, Edward A.; Nash, Huw M.; Felsch, Jason S.; Pal, Kollol; Lenz, George R.
PATENT ASSIGNEE(S): Neogenesis Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 139 pp.
CODEN: PIXXD2
DOCUMENT TYPE: **Patent**
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002020436	A2	20020314	WO 2001-US27226	20010831
WO 2002020436	A3	20030109		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001088617	A5	20020322	AU 2001-88617	20010831
US 2002077491	A1	20020620	US 2001-943852	20010831
PRIORITY APPLN. INFO.:			US 2000-230122P	20000905
			WO 2001-US27226	20010831

OTHER SOURCE(S): MARPAT 136:247586

AB The invention relates to methods for forming combinatorial libraries. The invention provides methods suitable for the rapid and convenient synthesis of very large combinatorial libraries of small org. mols. In particular, the invention provides a method for forming combinatorial libraries combining amide bond formation with epoxide opening. A method of forming a combinatorial library of compds. comprises reacting a plurality of core mols. (epoxides) with a mixt. of nucleophilic building blocks (amines) in a reaction vessel to form a library of compds., wherein each of said core mols. comprises (i) an acid halide, sulfonyl halide, isocyanate or isocyanate equiv., or activated ester functional group; and (ii) an epoxide functional group. More specifically, the method comprises sequentially (i) contacting the core mols. (epoxides) with a mixt. of amine building blocks so that reaction with the acid halide or activated ester functional groups is achieved; and (ii) adding a Lewis acid so that reaction of the amine building blocks with the epoxide functional groups is achieved. A total of 14 core epoxides, e.g. (RS)-, (R)-, or (S)-3-(glycidyloxy)isoxazole-5-carboxylic acid pentafluorophenyl ester (I), 1-glycidylimidazole-4,5-dicarboxylic acid bis(pentafluorophenyl) ester, 3-(glycidyloxy)-4-methoxybenzoic acid pentafluorophenyl ester, 4-(glycidyloxy)quinoline-2-carboxylic acid pentafluorophenyl ester, lactam (II), and spiroepoxide (III) were prepd. Thus, to a soln. of core epoxide compd. (RS)-I (100 mg, 0.28 mmol) in CH2Cl2/THF (3 mL each) at 24 .degree.C was added a soln. of amine building blocks (0.019 mmol each),

e.g. N-(2-chlorophenyl)piperazine and (R)-1-(4-methoxyphenyl)ethylamine, as a soln. in CH2Cl2/THF (3 mL each) and an Yb(OTf)3 catalyst soln. (100 .mu.L of a 120 mg soln. in 1.5 mL THF) and DIEA (50 .mu.L, 0.28 mmol) were added. The mixt. was heated to 45-50.degree. for 24 h, then cooled, treated with Amberlite (100 mg), stirred for an addnl. 1 h at 24.degree., and then filtered and concd. to yield the soln.-phase library as a slightly yellow film contg. 512 substitutionally and stereochem. unique compds., e.g. (IV) and (V). The library was screened by **automated ligand identification system** (ALIS) screening of E. coli dihydrofolate reductase (DHFRec).